

Figure 1

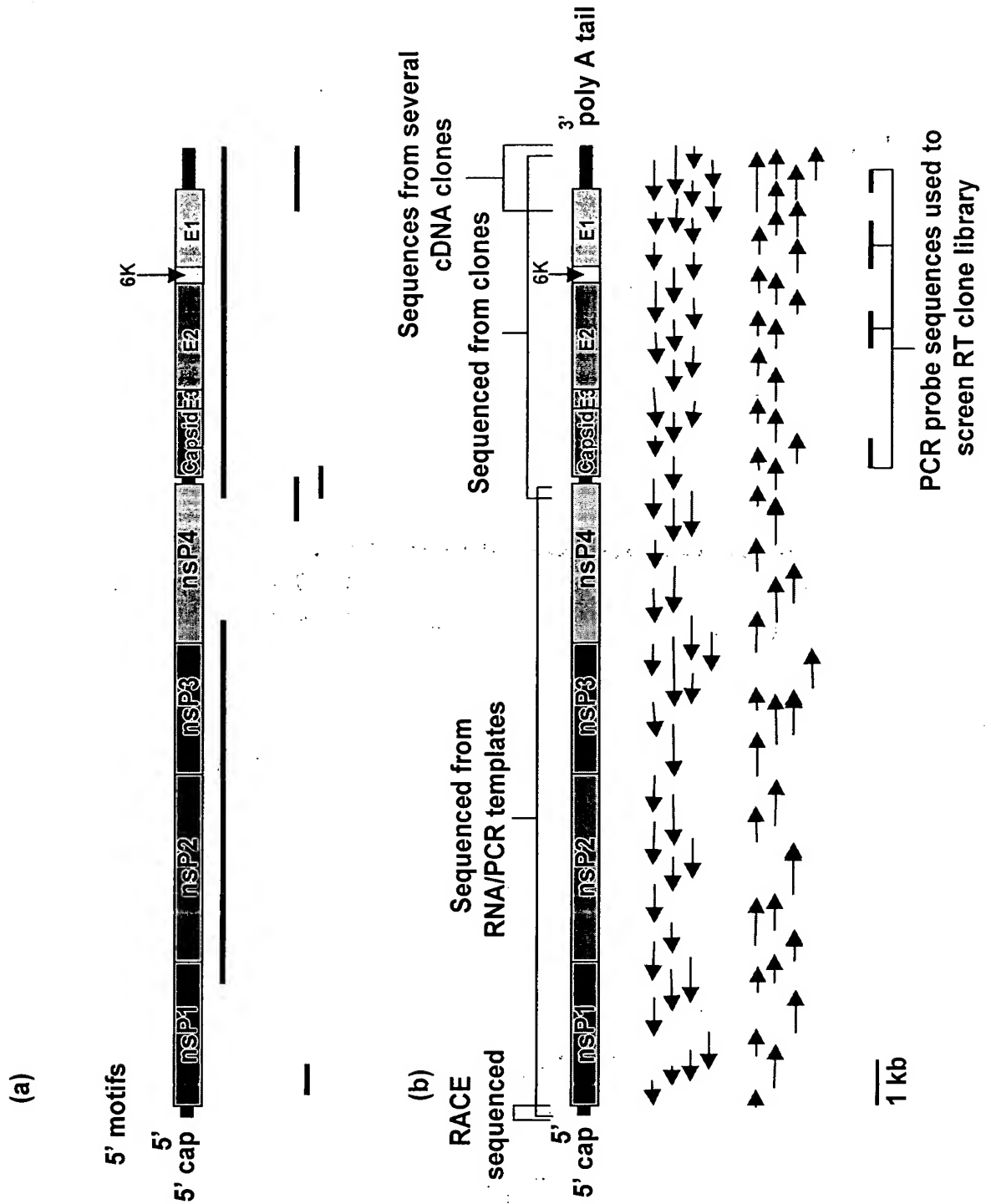
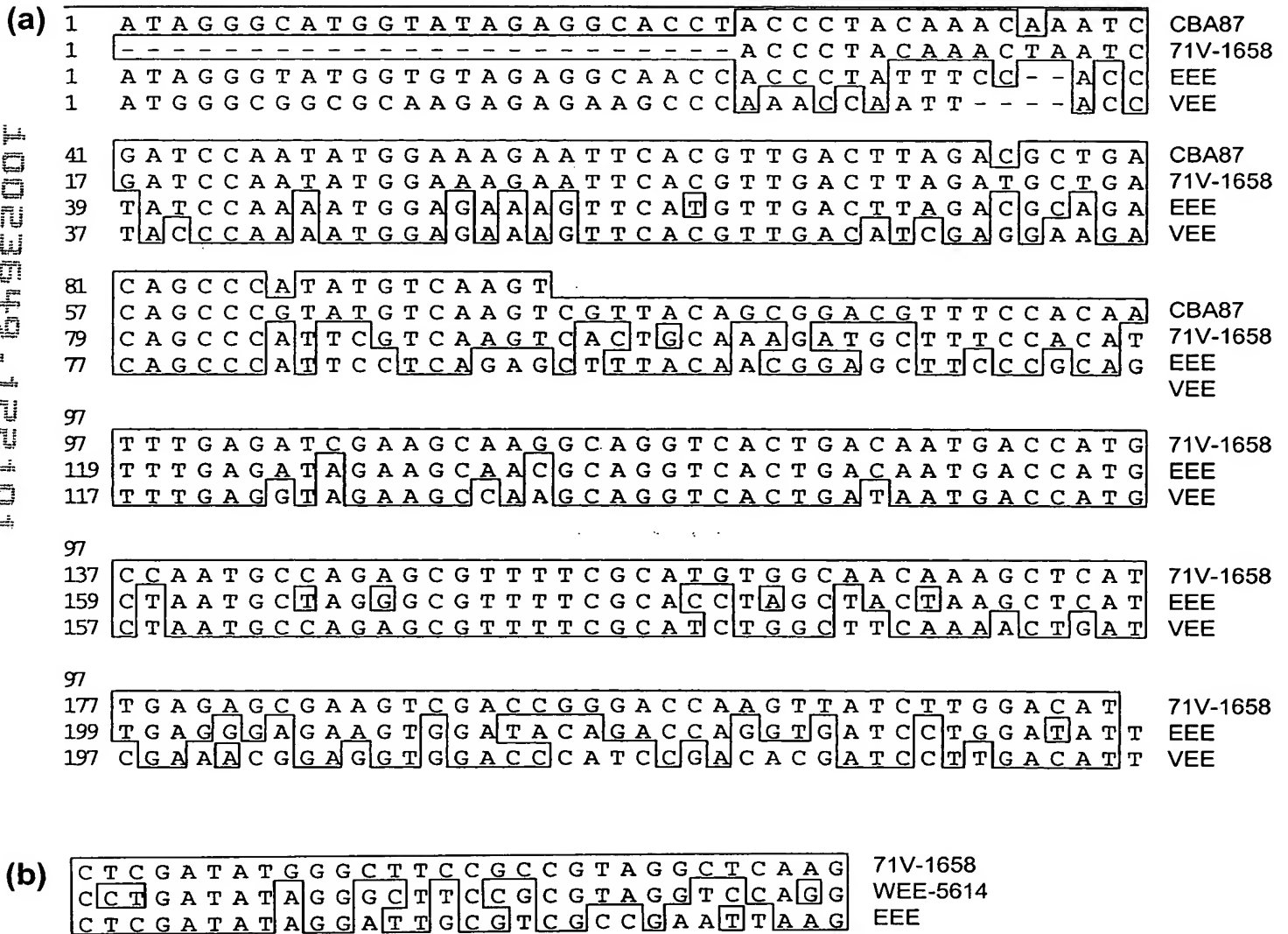


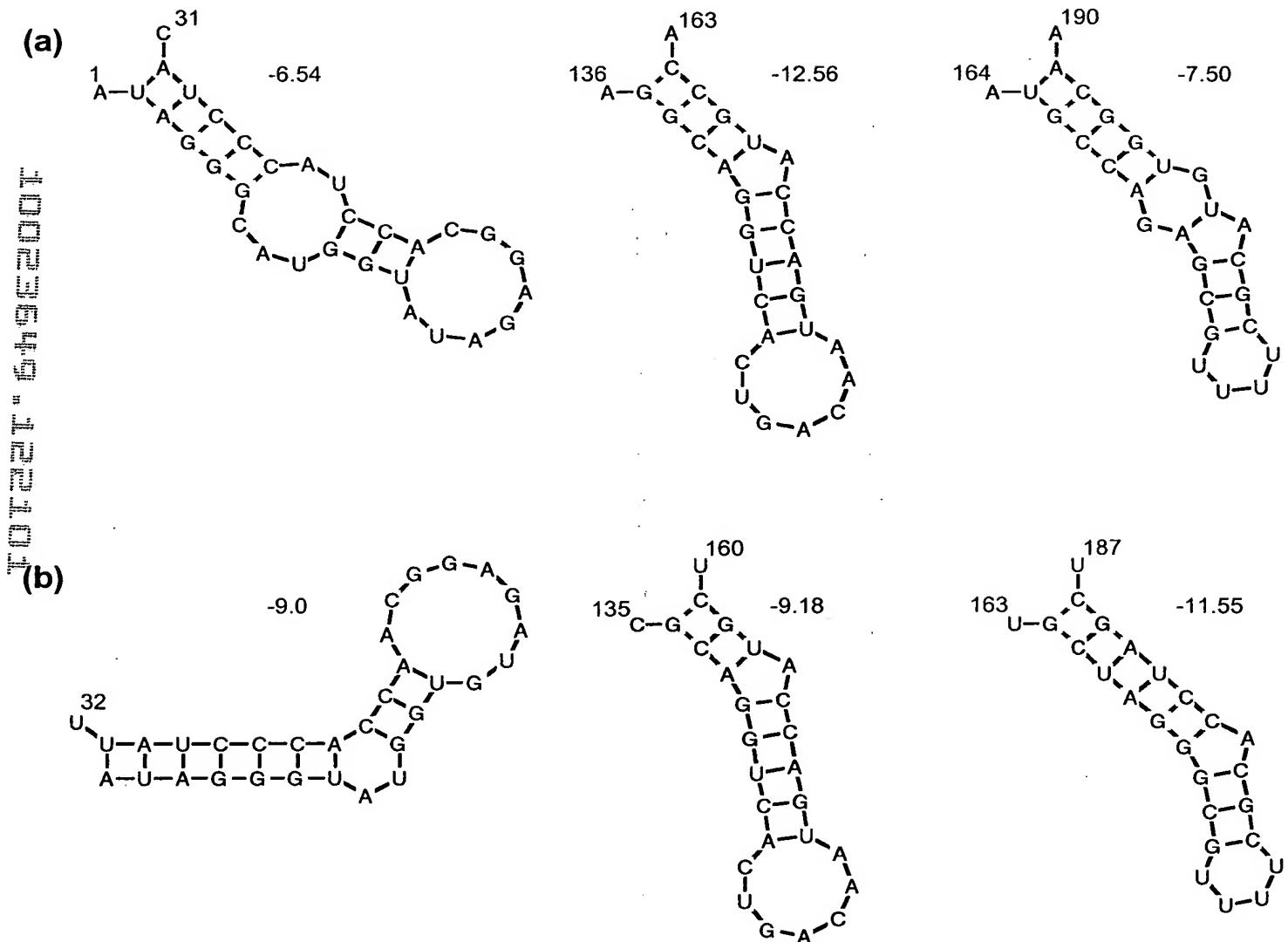
Figure 2 Multiple sequence alignment



a. The 5' terminus of WEE CBA87 (1-97), WEE 71V-1658 (25-240), EEE (1-238) and VEE (1-236) via Clustal module of DNASTar. Areas where sequences differ are boxed.

b. Hypervariable region identified in nsP1. Alignment of WEE 71V-1658 (1420-1449), WEE 1654 (65-94) and EEE (1415-1444) is shown.

Figure 3 Stem loop structures in the 5' NTR

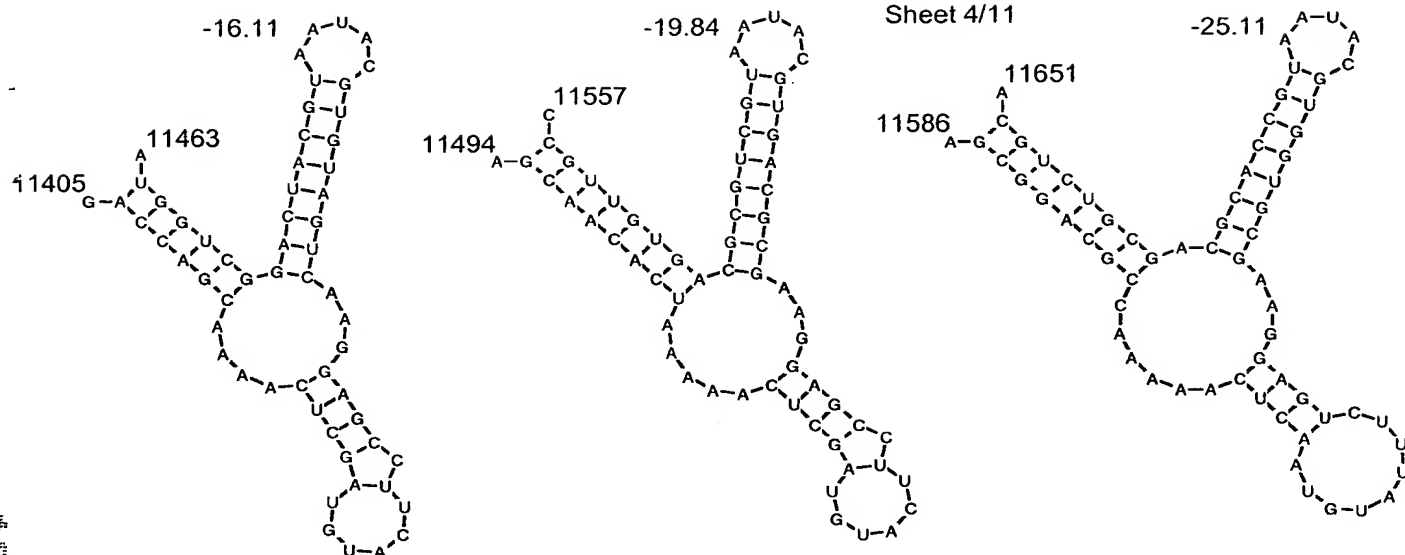


Hairpin structures were identified using the RNA folding program of the Genequest module (DNASTAR).

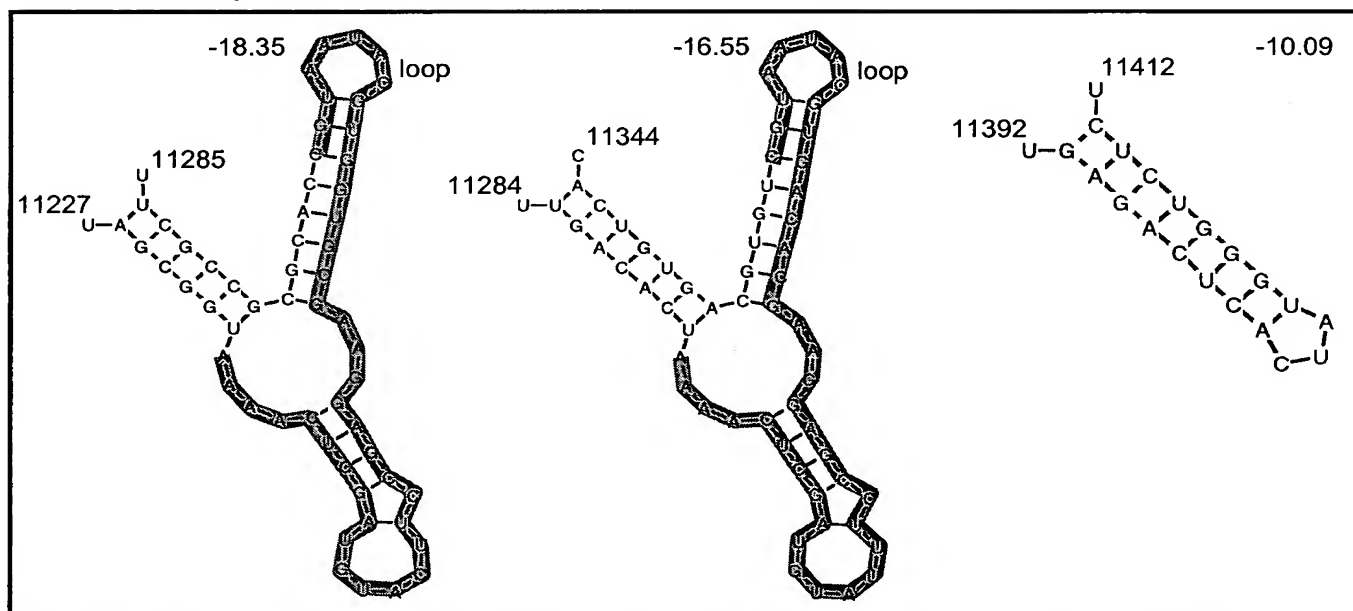
a. Structures for WEE (CBA87/71V-1658) sequence (1-192).

b. Structures for EEE (1-192).

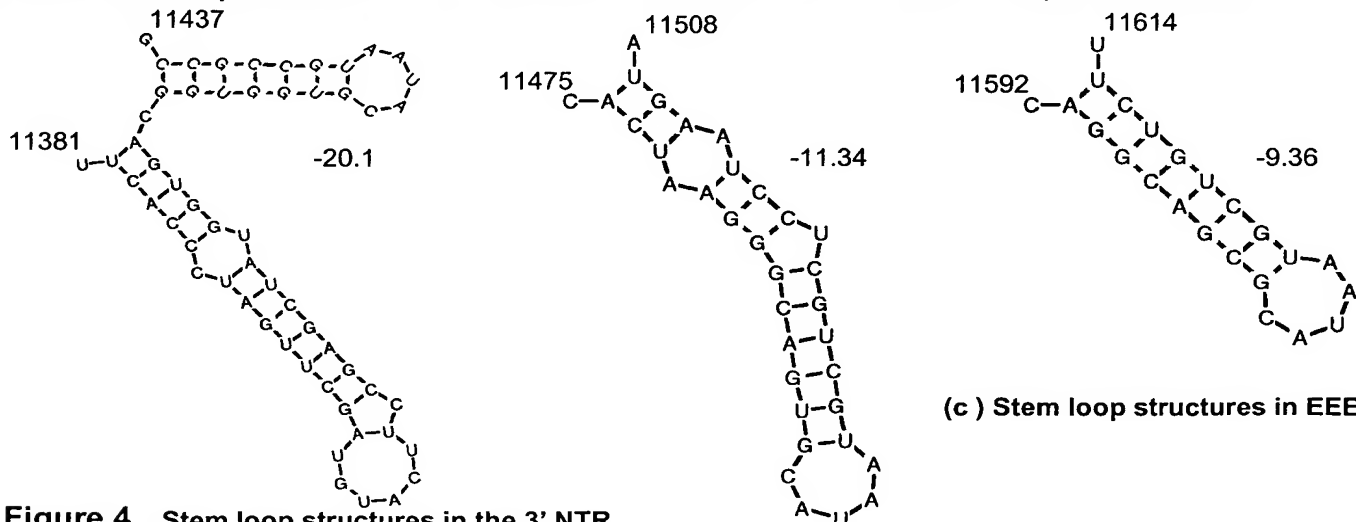
Minimal free energy values are shown for the different structures.



(a) Double stem loop structures in SIN.



(b) Double stem loop structures in 3' NTR of WEE. Residues in the SIN-like 40 nt repeat are shaded.



(c) Stem loop structures in EEE.

Figure 4 Stem loop structures in the 3' NTR

Figure 5 Phylogenetic relationship of the WEE nonstructural region compared to other alphaviruses

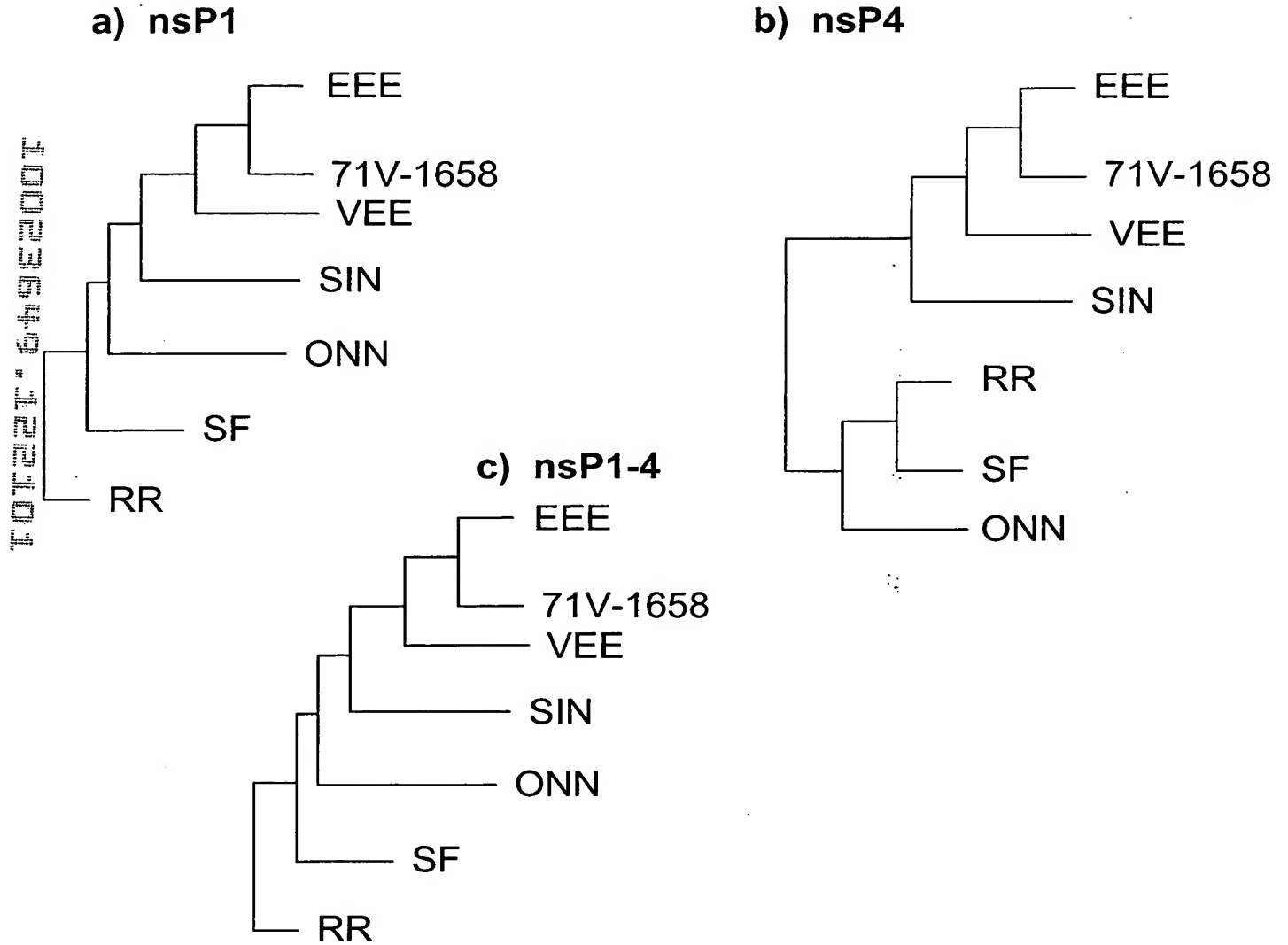
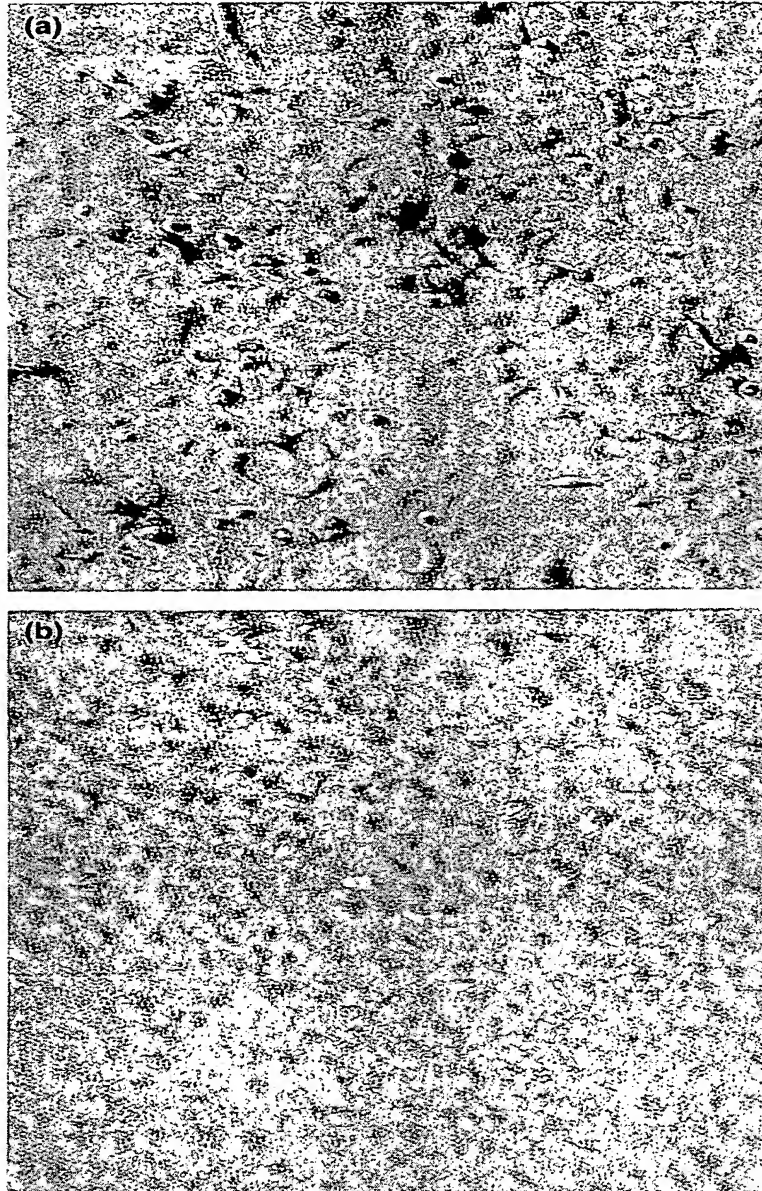


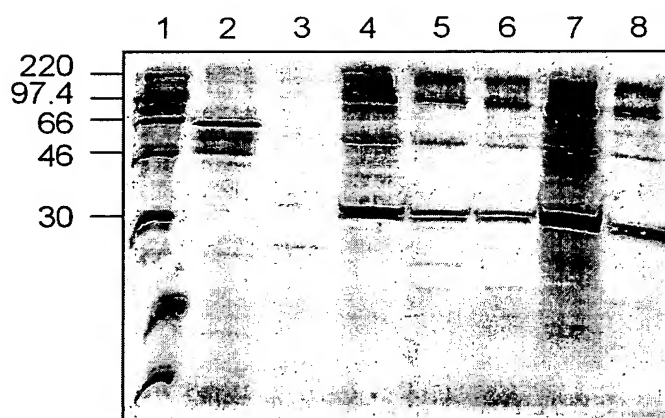
Figure 6 Expression of WEE structural genes in cell culture



One μg of plasmid DNA was transfected into Vero cells. After 31 hrs incubation, the cells were histochemically stained using a monoclonal antibody to WEE (11D2).
a. pCXH-3; b. pCI (control plasmid).

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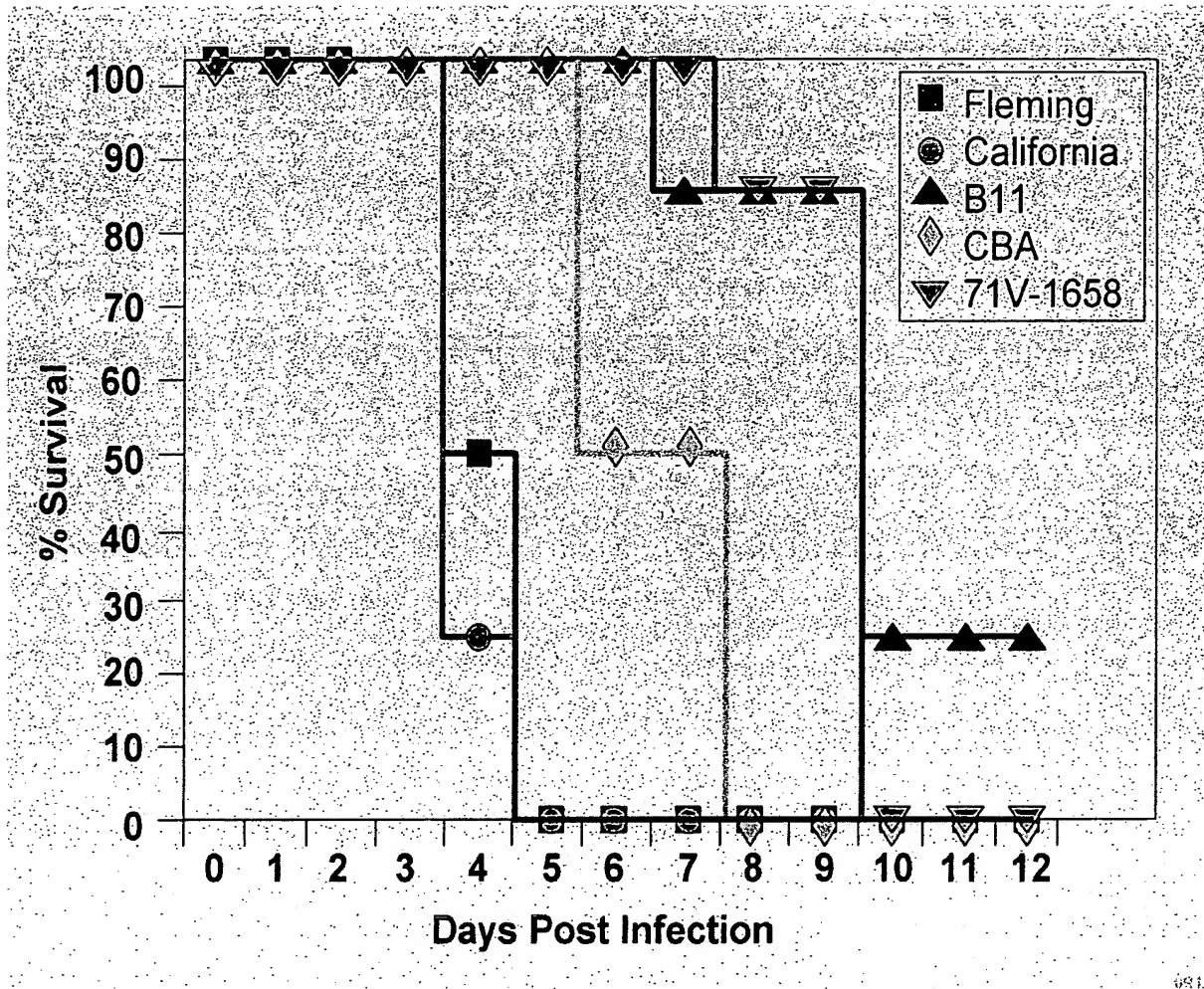
Figure 7 In vitro transcription and translation of WEE expression vectors



Qiagen purified vectors containing the WEE 26S insert were expressed *in vitro* using the TNT system and [³⁵S]-methionine labelling. Three μ L aliquots of each samples were run by SDS-PAGE on a 12% gel.

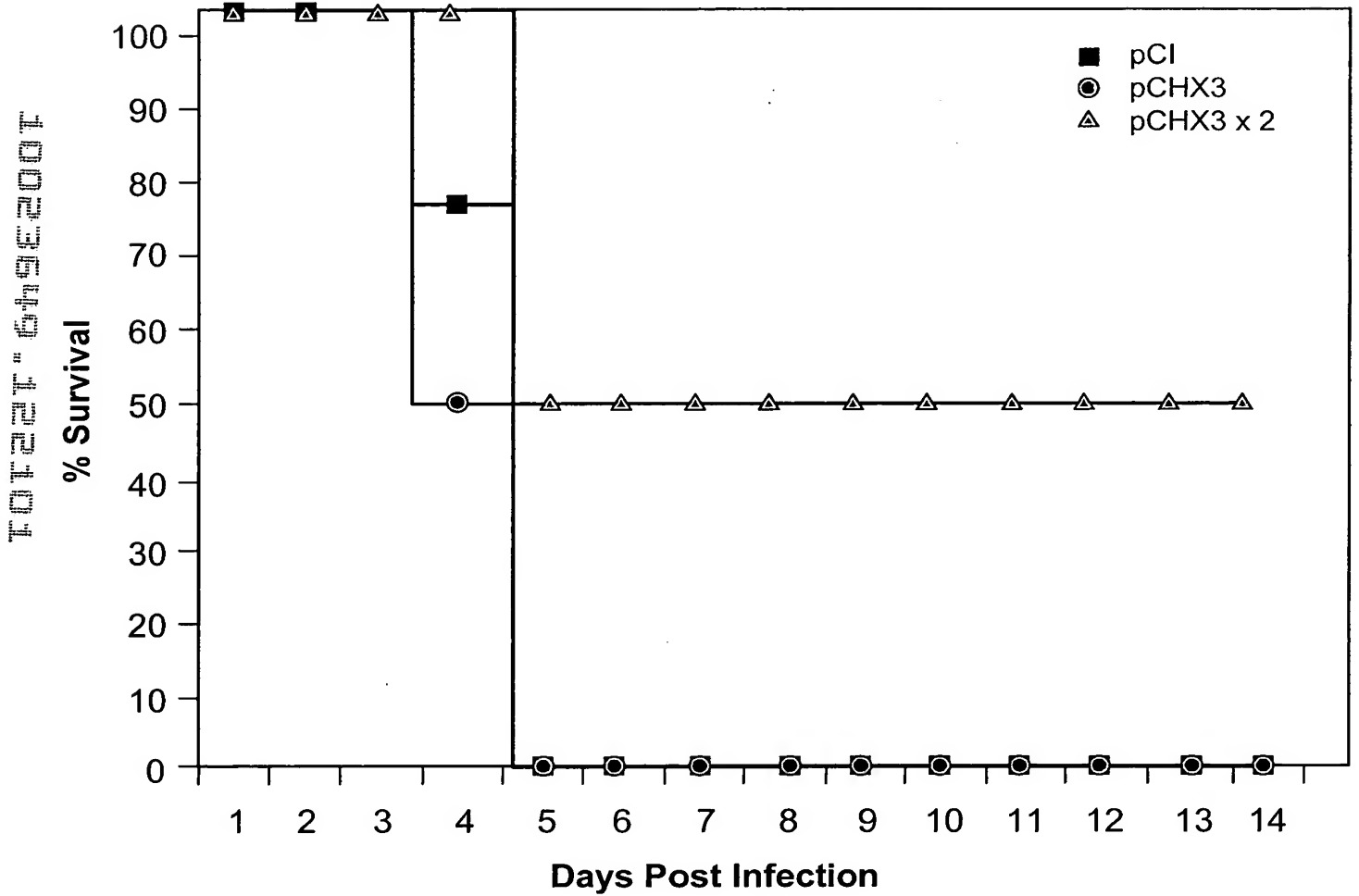
Lane: 1) Rainbow ¹⁴C-labelled marker; 2) Luciferase translation control; 3) pVAX; 4) pVHX-6; 5) pCXH-3; 6) pcDWHX-7; 7) pcDWHX-45; 8) pXTR2-4.

Figure 8 WEE mouse infectivity model



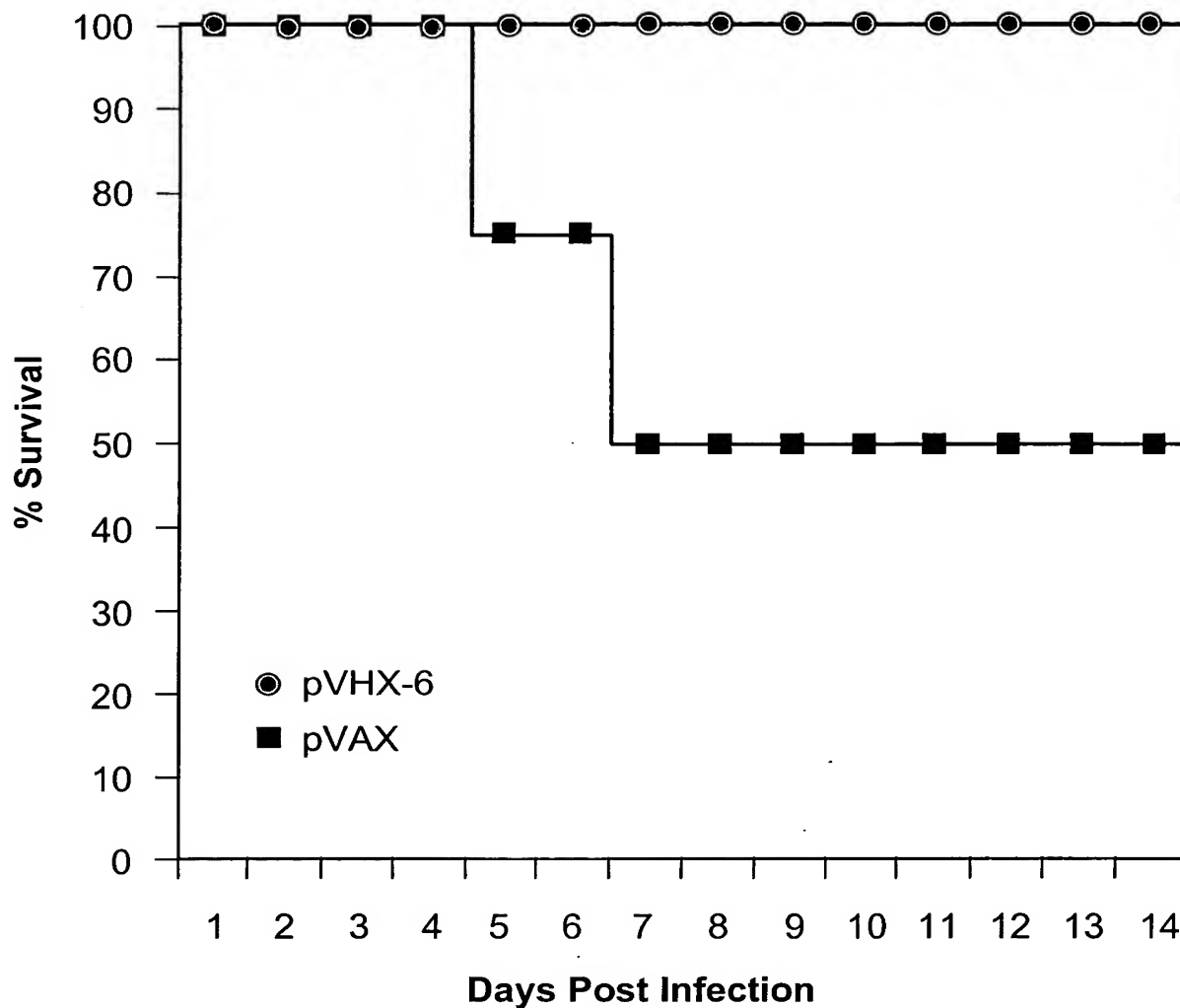
Groups of 4 mice were inoculated intranasally with 50 μ L of virus (approximately 10^4 PFU). The mice were monitored for 12 days, and the % survival graphed.

Figure 9 Protection using ballistic delivery of pCXH-3



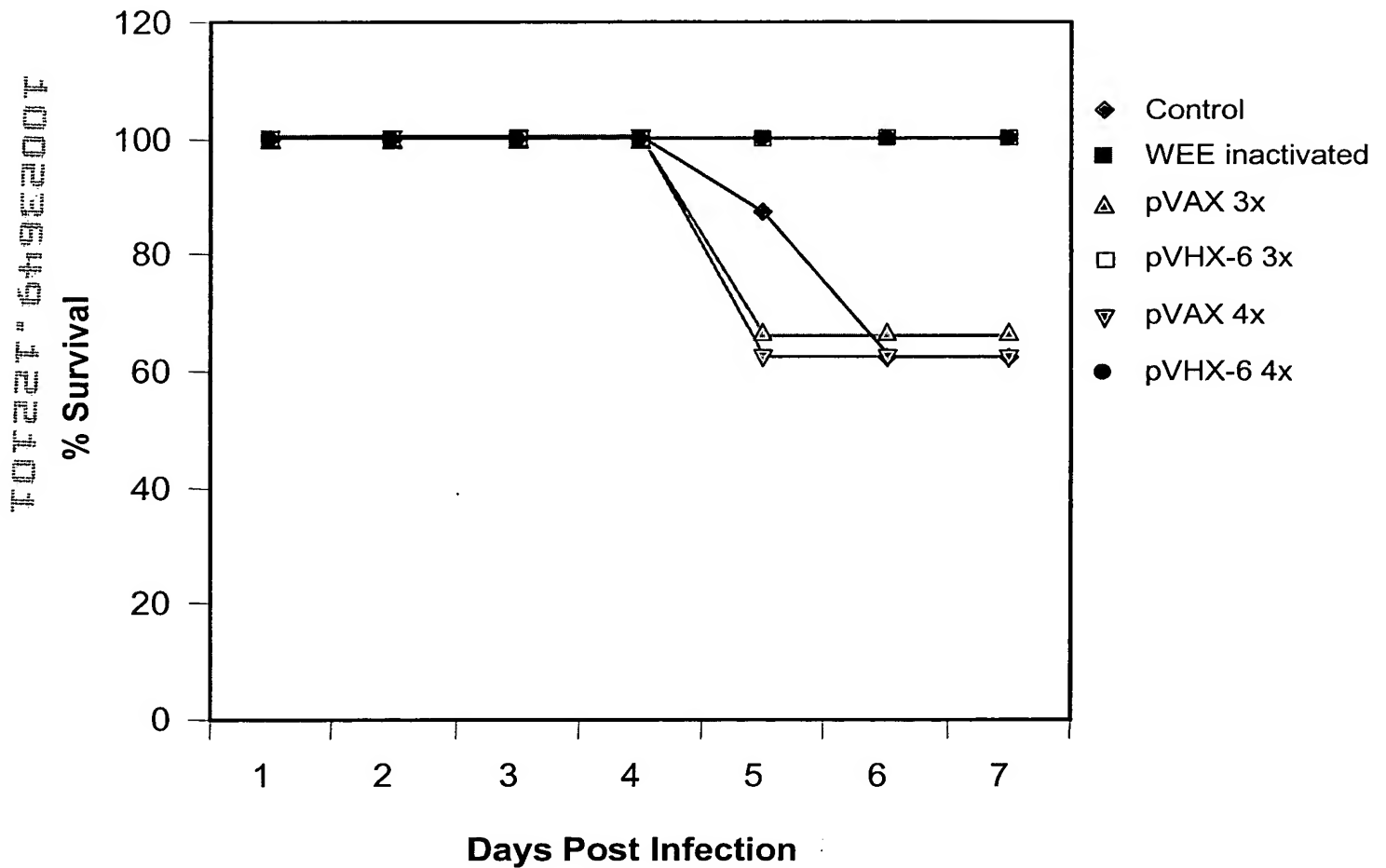
Groups of 4 mice were immunized with one or two doses ($2 \times 1.25 \mu\text{g}$) of either pCI or pCXH-3. The interval between boosters (2 doses) or challenge was 3 weeks. The mice were challenged intranasally with $50 \mu\text{L}$ of WEE Fleming (1.25×10^4 PFU). The mice were monitored for 12 days, and the % survival graphed.

Figure 10 Protection using ballistic delivery of pVHX-6



Groups of 4 mice were immunized with four doses ($2 \times 1.25 \mu\text{g}$) of pVAX or pVHX-6. The interval between boosters or challenge was 2 weeks. The mice were challenged intranasally with $50 \mu\text{L}$ of WEE Fleming (1.25×10^4 PFU). The mice were monitored for 14 days, and the % survival graphed.

Figure 11 Protection using ballistic delivery of pVHX-6



Groups of 5-8 mice were immunized with three or four doses ($2 \times 1.25 \mu\text{g}$) of pVAX or pVHX-6. The interval between boosters or challenge was 2 weeks. The mice were challenged intranasally with $50 \mu\text{L}$ of WEE Fleming (1.7×10^4 PFU). Untreated control and WEE inactivated control (3 doses) groups were also included. The mice were monitored for 14 days, and the % survival graphed.